NUS_Singapore Business Plan for iGEM 2016

1. Background

1.1. Profile, Vision and Mission

NUS_Singapore is a team of students from the National University of Singapore (NUS) interested in using synthetic biology to design solutions for the detection of cancers. Our team created a spatially controlled cancer detection system using engineered *Escherichia coli* in order to increase specificity to the target cells and possibly concentrate the drug payload at the intended site. Our engineered bacteria makes use of two sensors to detect cells causing disease and sense specific stimuli present in their environment for more specific and accurate cancer detection. The spatial markers were specially chosen to circumvent a common problem in cancer research, where heterogeneity in different types of cancers results in the lack of a specific diagnostic for a variety of cancers.

Our vision is to maximize the specificity and minimize the toxicity of cancer diagnostics in order to improve efficacy and accuracy of the diagnosis.

Our mission is to innovate an affordable cancer diagnostic system with high specificity and effectiveness. We foster interdisciplinary research within a conducive environment and utilise various platforms to bring our products to the market efficiently.

1.2. The product

Our product, named the Regulated Invasive Organism Targeting System (RIOT System), employs a modular concept of integrating different genetic circuits that serve individual functions with crosstalk capability. The current system consists of three parts: The RIOT Sensor, RIOT Responder, and the RIOT Invader.

At the initial stage, the efficiency of our product is tested using cultured cancer cells as a model. The engineered *E. coli* targets cancer tumours by detecting molecules unique to the tumour microenvironment and a specific surface marker on cancer cells. Our bacteria-based diagnostic targets CD44 variant 6 (CD44v6) marker on the surface of cancer cells and senses high lactate concentration in their surroundings.

The RIOT Transponder is the first component of our RIOT system which targets the surface receptor CD44v6. This cell surface marker has been previously shown to act as a biomarker for cancer progression (Brabletz *et al.*, 2005) by interacting with growth factors and other factors which promotes migration and invasion of cancer cells (Orian-Rousseau, 2010). Our proposed RIOT Transponder consists of an anti-CD44v6 antibody, which binds to CD44v6 on cancer cell surface membranes, conjugated to a HasA hemophore protein. The hemophore module, when loaded with heme, serves as a signal to activate the RIOT Responder circuit within the RIOT bacteria. In this manner, the RIOT Transponder functions as a biomolecular bridge between the RIOT bacteria and the target cancer cells expressing CD44v6.

The RIOT Responder circuit consist of constitutively expressed genes of the haem acquisition system (HAS) pathway from *Serratia marcescens (ref)*. The circuit receives input from RIOT Transponder and initiates expression of the *luxR* gene under control of a HAS promoter (*pHas*). This expression output activates the RIOT Invader module which initiates an invasion of RIOT bacteria into the target cancer cell. Comment: Do you want to show the big picture from the website?

The RIOT Sensor module comprises of a biosafety switch under control of a lactate sensing promoter. The tumour environment of cancers such as breast cancers (Morais-Santos *et al.*, 2015) and cervical cancers (Walenta *et al.*, 2000) contains a high lactate concentration due to the Warburg effect. This is a common phenomenon where cancer cells have the ability to increase glucose uptake and undergo aerobic glycolysis to support their excessive growth, resulting in the elevated production and accumulation of lactate (Chen *et al.*, 2007; Vander *et al.*, 2009; Alfarouk *et al.*, 2015). With the knowledge of the Warburg effect, we introduced a biosafety switch under lactate sensor into bacteria so that they will not be able to survive in healthy tissues where the lactate concentration is below the threshold for the expression of the biosafety switch.

In summary, when our engineered bacteria are introduced into the tumour mass, high lactate levels will inactivate the biosafety switch. Hence, bacteria will survive and target cancer cells expressing CD44v6. This delivery system will increase specificity to the target cells and allow for accurate detection of the cancer cells. Additionally, a drug payload can be released at the intended site if needed. It will also potentially be a cost-efficient method as it can be adapted to treat a wide range of diseases according to the surface markers and environmental sensors specific to that disease.

2. Cancer Detection Using Bacterial Vector: The Need and Product Analysis

1.3. The Need

Our spatially controlled cancer diagnostic system using engineered bacteria offers an attractive opportunity for sales within the industry. There are many beneficial factors in the industry supporting the development of our targeted drug delivery product. According to the World Health Organisation in the World Cancer report 2014, the annual number of cancer cases will rise from 14 million in 2012 to 22 million in the next twenty years. This inevitably creates the demand for safe, cost-effective and prompt cancer diagnostic tools, making the cancer diagnostic market a profitable industry to enter (Figure 1).

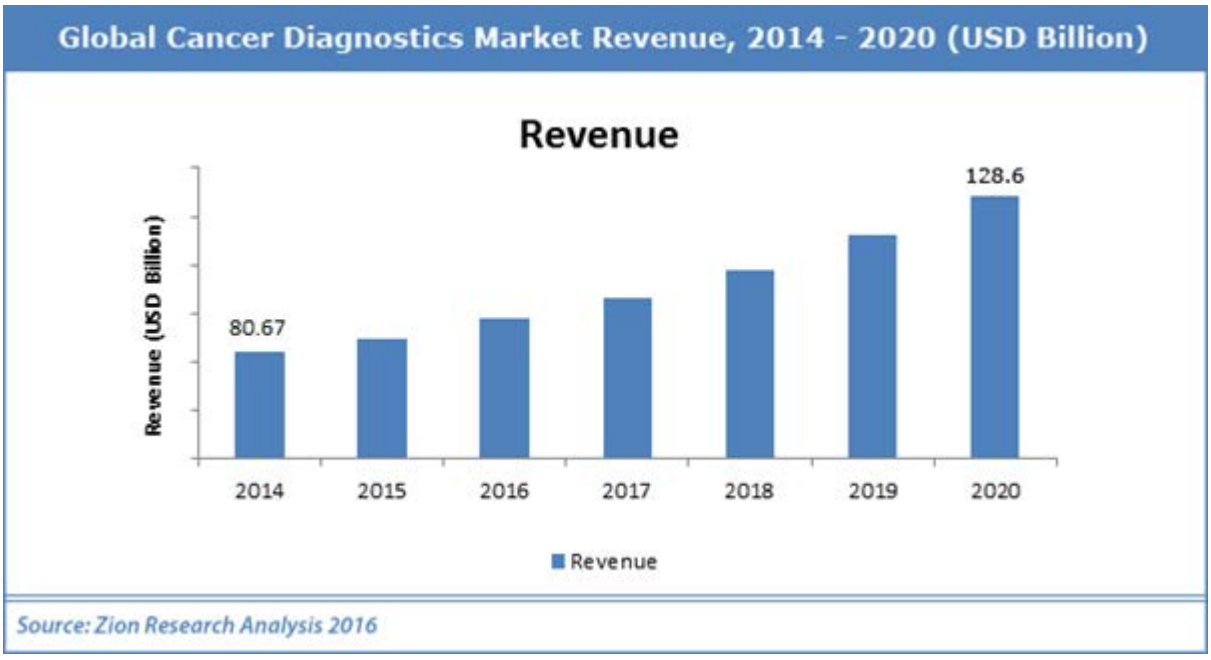


Figure 1: Current and projected growth of the cancer diagnostics market revenue. Taken from Zion Research Analysis 2016.

Secondly, the advancement of spatially and temporally controlled systems, as well as the discovery of many disease biomarkers drives the development of bacterial diagnostic systems. Using disease biomarkers, the specificity of our diagnostic system is enhanced. Furthermore, more modules that detect a larger number of disease biomarkers can be added to our system, as well as an additional drug payload if needed. The flexibility of our system allows us to modify the target of our diagnostic system, therefore making it viable across different diagnostic markets, not just cancer.

Thirdly, the intellectual property protection in Singapore supports strategic partnerships opportunities for pharmaceutical companies and research institutes. This helps to secure the financing required for further development and maintain the competitive advantage for companies, as well as creating values for their stakeholders. Besides, many government initiatives such as the Translational & Clinical Research Flagship Programme, the Competitive Research Programme and the Health Services Research Competitive Research Grants have provided fundings and support to enable researchers to devote time

and effort to research activities. In short, we believe that the industry is ready for the entry of our product which has great potential to capture a share of the drug delivery market, hence creating a valuable opportunity for business growth.

1.4. Potential customers

With a strong foundation in biomedical sciences, manufacturing and R&D activities, Singapore offers us various public-private partnership opportunities. The following table provides names of potential customers which can be further expanded as our team develops a network of contacts.

Table 1: Potential customers.

Research institutes	The Institute of Bioengineering and Nanotechnology (IBN) is a biomedical science institute in Singapore. One of the areas of research at IBN is the development of biosensors and bio devices to improve healthcare and the quality of life.
Clinical-research units in hospitals	Hospitals like Singapore General Hospital usually engages in scientific research to improve clinical outcomes.
Pharmaceutical and biotechnology companies	Restalyst is a privately-owned biomedical company that is based in Singapore, focusing on the development of new diagnostics using existing and novel biomarkers. Dx assays Pte Ltd offer highly customizable services in areas such as infectious diseases, oncology, genetic testing and companion diagnostics using biomarkers. Nova Satra Diagnostics specialises in research, development and commercialization of blood-based diagnostic tests that provide the early highly accurate detection of cancer.

1.5. SWOT (Strengths, Weakness, Opportunities and Threats) analysis

S - Team's member has diverse academic background and laboratory experience. - Cost-effective manufacturing by culturing and harvesting the engineered bacteria. - Low level of competition from commercial products in Singapore market which provided us excellent sales opportunity.	W - Bacteria used in in-vivo diagnosis or drug delivery are based on expression systems that used antibiotic resistance gene as selection marker which may be transferred to intestinal microbiota. Regulatory measures should be considered to ensure the safety of the used strains. - Immune response caused by bacterial proteins - Lack of established sales channel
O - Readily market for disease diagnosis. - Possibility to diagnose and treat wide ranges of diseases by using biomarkers specific for that disease.	T - Potential competitors in international market

3. Marketing strategy

3.1 Aims of the Marketing Team

Our main marketing strategy stems from results obtained from a pilot survey administered in schools and amongst other members of the general community in Singapore. Our target was to identify the areas in which perception of the RIOT System was or was not favourable and could be improved on, whether in terms of outreach or by word of mouth promotion. We also carried out this survey in other countries in the Asia-Pacific region, and these surveys will be discussed under Section 3.5 (International Expansion).

By analyzing the responses obtained from these surveys, we will get a clearer idea on which aspects to better improve the marketing of our product and make it appeal to the masses. This report will focus on two key aspects of which the outlook of the system can be improved upon: the perception of the general public towards the RIOT System, and how safe they deem it to be.

3.2 Marketing Survey Results

Our target population in the survey held across communities in Singapore achieved a total of 163 responses in the following proportions, as shown in Figure 2:

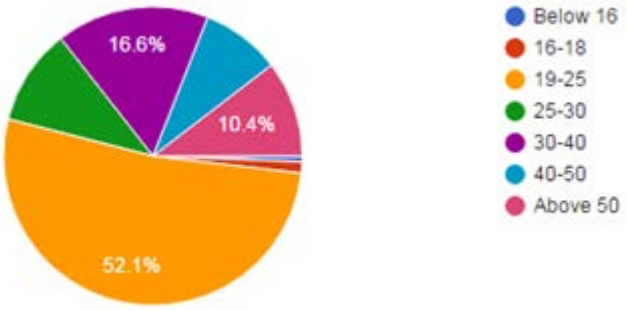


Figure 2: Distribution of responses in the survey locally administered within Singapore.

As such, any measures we would potentially be taking to address the issues of receptivity and safety of the system should be duly catered to the age groups providing the majority of responses – to the community between the ages of 19 and 25. The abovementioned two areas of concern had the following distributions of responses (Figure 3):

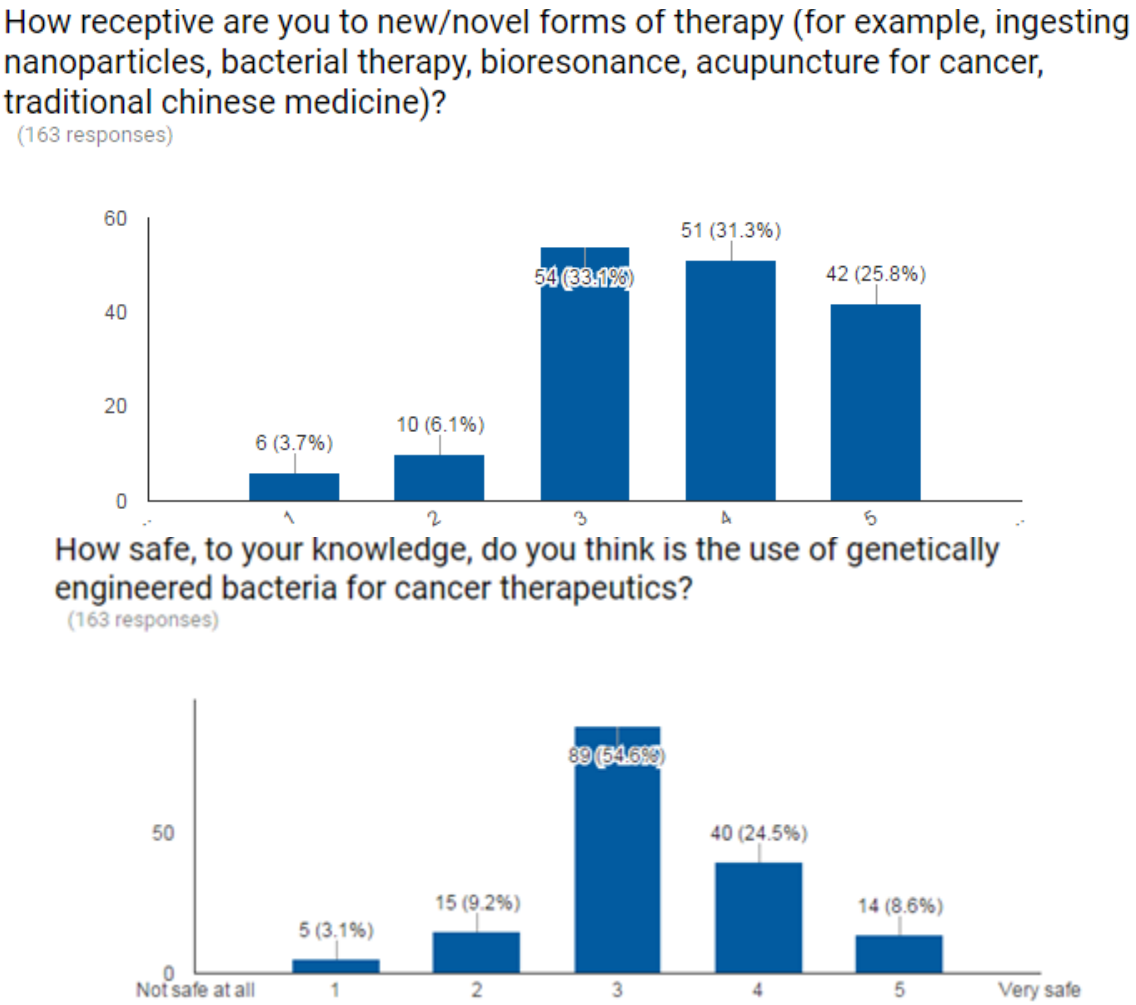


Figure 3: Distribution of responses in a survey on receptivity (top) and safety (bottom) perceptions with regards to the RIOT System.

In Figure 3, respondents were asked the questions as seen and rated their response on a scale of 1 to 5, where 5 is very receptive or very safe, and 1 is not receptive or not safe at all. While the majority of the respondents show receptivity to novel forms of therapy and 25% of respondents are completely receptive to it, conservative values are obtained when asked about the safety of bacterial use in cancer therapeutics, with only 8.6% who feel that the system is very safe. We begin to see that perhaps, safety is the bigger issue that is hindering the public from accepting the RIOT System.

3.3 Analysis of Survey Results

With the raw data now available, it is important that we analyse this data statistically to ensure that we spot trends in how receptivity and safety could be connected to one another. By spotting this trend, we will be able to uncover which is the true source of hesitance from the public and tackle that with a specific marketing plan.

Upon analysis of the above components, we see the following pattern:

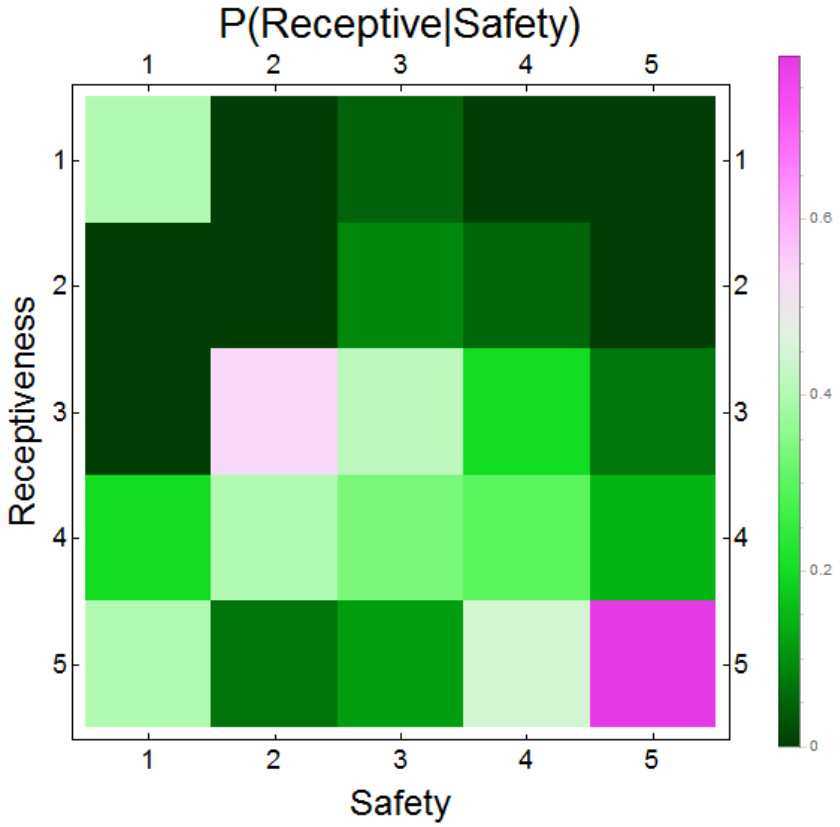


Figure 4: Trends of probabilities of the level of safety given the reported level of receptiveness.

Figure 4 illustrates the probability of a certain level of safety given the corresponding level of receptiveness the participants have indicated. It is clear that participants who thought the RIOT System is completely safe (denoted by a rating of '5') are also noted to be highly receptive to the treatment, as denoted by the bright pink zone on the bottom right corner. Also, we can see that despite the levels of safety indicated by participants, there are many participants who have indicated their receptiveness levels between 3 and 5. This means that regardless of how safe the participants deem the RIOT System to be, they seem to be receptive to the idea of such a diagnostic tool.

Therefore, the Marketing team believes that there is a demand for such a tool, and strengthens the need to market the RIOT System to our potential customers. We deduce that dealing with the safety perception of the system would be the ideal way to prove to the community on the viability of the tool and ensure its promotion to them.

3.4 Proposed Mode of Action – Marketing

To ensure that potential customers are aware of the safety aspects of the RIOT System, an outreach programme is necessary to carry the message to our target groups, earlier identified from filtering the survey responses. As we are catering mainly to the ages between 19 and 40, we can establish a two-pronged approach:

Outreach Programme in Universities	**Family-based Workshops**
❏ Seminars in the university setting will be paramount in getting the message to all students, not only those in Biology-related fields ❏ An introduction to how the RIOT System is able to specifically detect cancer cells using cancer cell markers would be the main theme of the seminar ❏ Key aspects of safety (immune response to injected bacteria, accidental release into the environment etc.) will be covered in the seminars, directed by professionals in the pharmaceutical and biotechnological firms.	❏ Based in a more familial setting (e.g. Community Centres (CCs) in suburban neighbourhoods which are frequented by patrons of all ages ❏ Particularly useful in reaching out to the older generations ❏ To convey our message in simpler terms, interactive sessions could be carried out to help the patrons visualise what the RIOT System is and how it will be safe in practice ❏ The simplified message from the CCs can then be more easily communicated to other family members.
● Potentially, companies with the Biopharmaceutical Manufacturers' Advisory Council (BMAC) may provide sponsorship and participating members for the seminars.	● A possible collaboration between the Marketing team and the Grassroots leaders of the People's Association of Singapore could be established, to integrate the programme and hold it in tandem with the others held in the CCs.

3.5 Post-Action Review (PAR) and International Expansion

It is vital that we ensure progress in altering the perception of the RIOT System has been made with our marketing efforts. To measure this, a PAR will be carried out after the first round of outreach programmes are complete. We will aim for the same categories for our target audience to see how much perceptions of safety has been altered in the preceding months.

If successful, we can consider an international expansion after financial consideration. After administering our survey to our collaborators in the University of Hong Kong (Hong Kong) and University of Melbourne (Australia), we obtained the following age, receptivity and safety perception distributions:

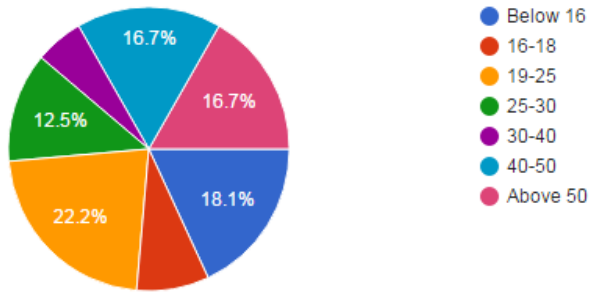
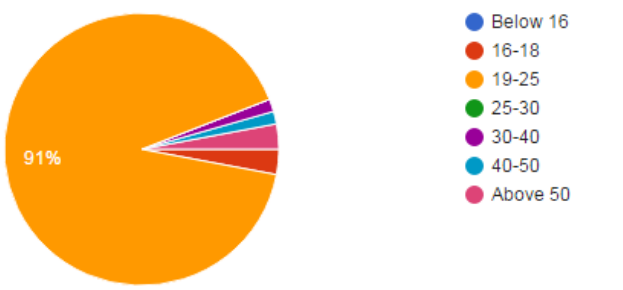
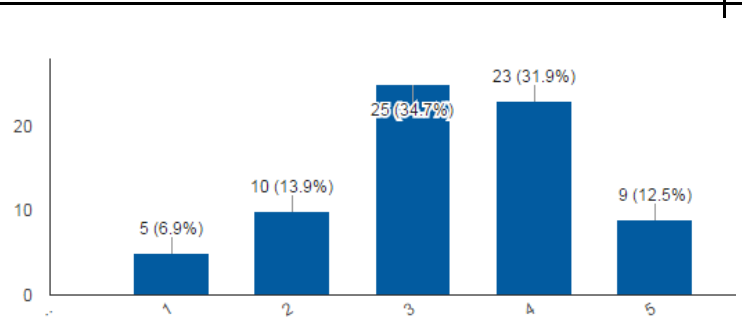
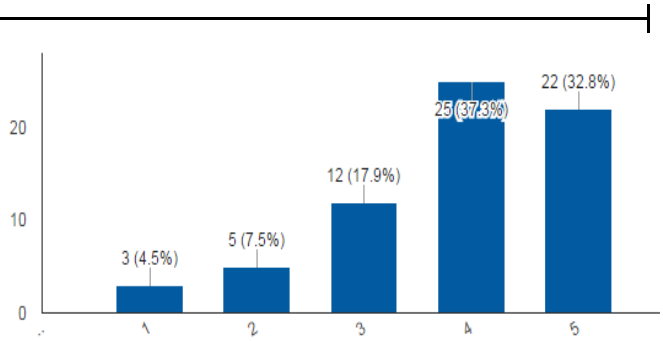
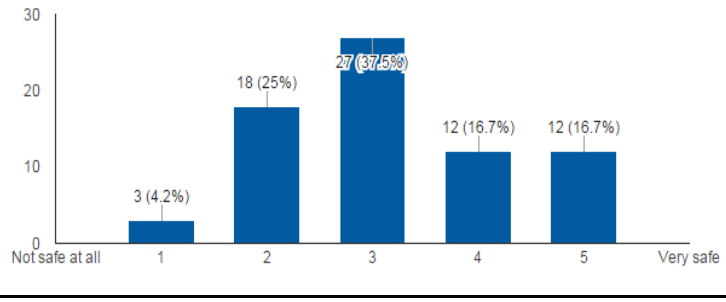
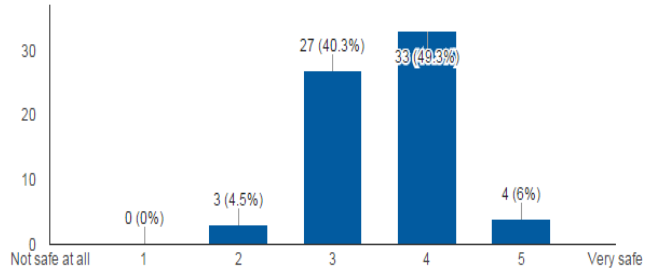
	Hong Kong	Australia
Age distribution		
Receptivity		
Safety perception		

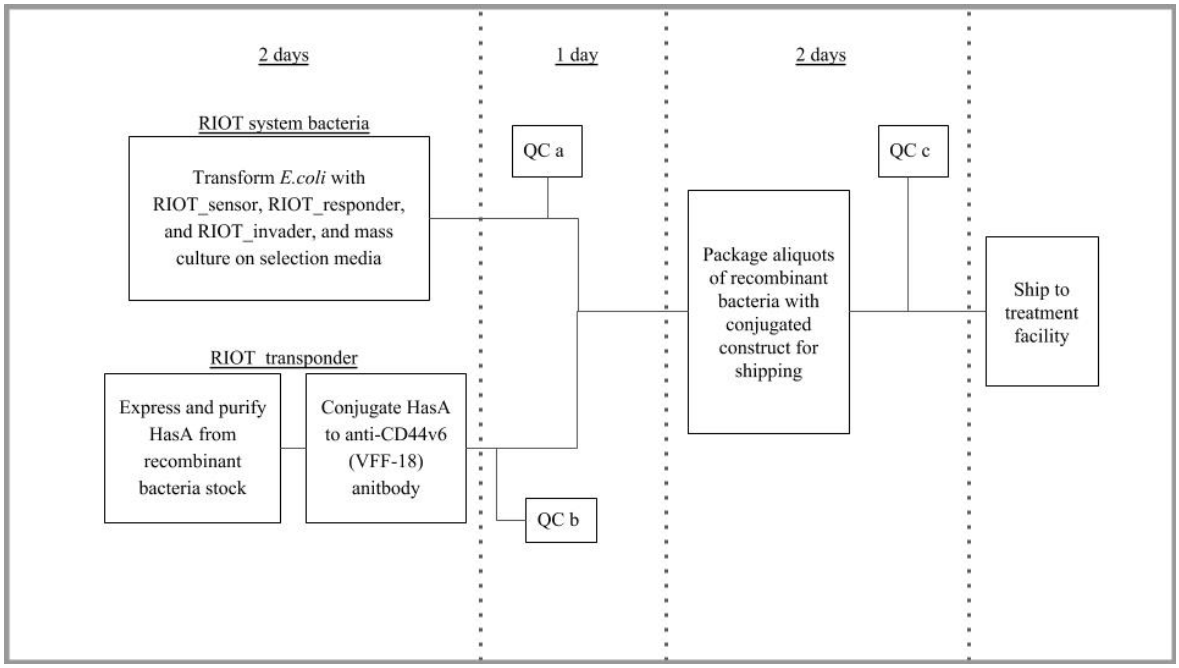
Figure 5: Distribution of responses in the survey in other countries

As we can observe, the receptivity and safety perception patterns seem to mirror those obtained from Singapore - participants are generally receptive to the RIOT System diagnostic tool but do not think it is a completely safe method of diagnosis. Hence, if the marketing programmes implemented in Singapore prove to be a success, a second collaboration with our partners could be established to promote the system in these regions with similar approaches.

4. Manufacturing and Operations

Timeline

- If all products are in stock, shipment can be delivered after 2 days provided QC checks are passed
- If products are not in stock, timeline of manufacture is as follows



RIOT system bacteria

The RIOT system bacteria is manufactured by transforming *E. coli* with the gene circuits for

- RIOT Sensor
- RIOT Responder
- RIOT Invader

Verification of transformation is carried out by Sanger sequencing. Expression of protein components of the gene circuits is verified by immunoblotting.

Storage: The RIOT System bacteria is kept at -80°C as 20% glycerol stock for up to 3 months.

RIOT Transponder

The RIOT Transponder antibody-protein conjugate is manufactured by expressing His-tagged HasA hemophore protein in *E. coli* BL21. The HasA is purified via metal affinity methods. The anti-CD44v6 (VFF-18) antibody is purchased separately from commercial sources. Conjugation of anti-CD44v6 (VFF-18) to HasA is carried out.

Storage: The RIOT Transponder can be mass produced and kept at -20°C for up to 6 months.

Shipment

- RIOT bacteria will be packaged in aliquots of 10^6 colony forming units (CFUs) in 10% glycerol per dose
- RIOT Transponder will be packaged in aliquots of 100 µL of 10^{-6} M to be diluted as necessary based on tumour size

All products are shipped in dry ice.

Quality Control

Prior to packaging for shipment, all products will have to undergo and pass QC checks as stated below.

QC a: RIOT system bacteria

- Extract random bacterial stock sample and sequence plasmid DNA to verify presence of RIOT gene circuits
- QC Check - extract random bacterial stock sample and conduct immunoblot to verify presence of
    - HasR
    - HasS
    - HasI
    - LuxR
    - Alanine racemase
    - Invasin
    - LLO

QC b: RIOT Transponder

- Verify purity of HasA by SDS-PAGE and Immunoblotting
- Verify conjugation efficiency by Immunoblotting

QC c: RIOT system

- Cell invasion assay of complete circuit

5. Environmental Impact

Currently, Singapore does not have a dedicated umbrella legislation specific for the regulation of GM technology and its products. However, GMAC (Genetic Modification Advisory Committee of Singapore) has till date only released two sets of guidelines covering the commercial release of agriculture-related GMOs and research on GMOs including its import. Therefore there are currently no laws specifically catering to the commercial use of genetically modified microorganisms (GMM) for therapy.

Since this company plans to sell GMMs (which will come in contact with the environment), the company will first have to submit the details in a 'Report on the Composition of the Institutional Biosafety Committee' form and subsequently await approval from The Institutional Biosafety Committee (IBC). Endorsement from the GMAC would also be required.

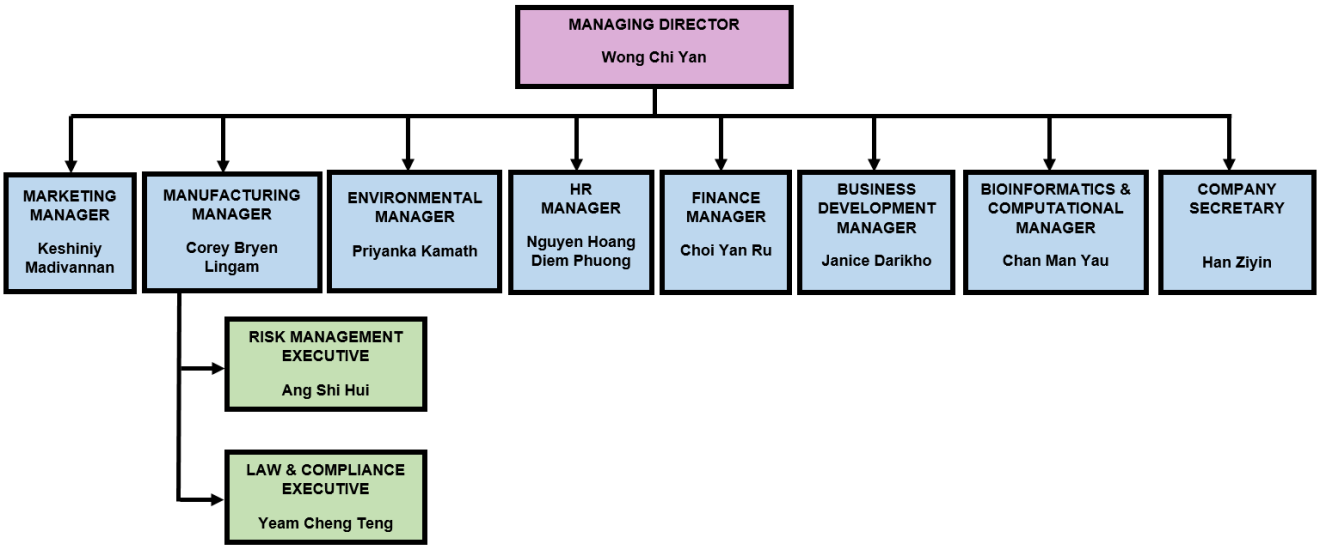
Our product involves introducing genetically engineered bacteria directly into the tumour mass. It is a target specific invasive therapy so chances of the engineered bacteria escaping into the environment are low. In the highly unlikely event that the genetically engineered bacteria does happen to escape into the environment, the bacteria will not survive long due to the lack of high lactate in the environment. It could however, survive in lactic acid containing products like yoghurt but if ingested, would not pose a threat because its survival chances would be negligible.

In addition to this, the company will ensure strict CIP (Cleaning In Place) operations during the industrial manufacturing process. Equipment will undergo steam sterilization and/or disinfectant wash periodically after usage. This will ensure minimal chances of the GMO escape into the environment.

6. Human Resource Management

These policies encompass basic principles for efficient and effective human resource management. We allow for flexibility and dynamic in our system so that it can be adjusted and adapted to changes in the local market, culture and legislation.

6.1 Organizational structure



6.2 Performance appraisal

We honor the service and dedication of our employees since they are the central factor to our success. In order to provide feedback on past performance and potential for future development, formal assessments are taken place on a semi-annual basis. Assessment and promotion decision are based on the agreed objectives and the employee's competence, skills, performance and potential with the exclusion of any consideration for origin, race, nationality, gender, religion or age.

Both the positive and negative feedback of individual performance should be taken place through open communication with written evidence. Feedback is based on mutual trust and willingness. The main purposes are to stimulate performance and encourage continuous improvement. Appropriate training on-the job and formal training programmes will be provided to improve relevant skills and competencies. Communication occurs at different levels via various means including using the internet, emails and weekly staff meetings.

6.3 Work-life balance

We believe that the employee's private and professional life should have a good balance. Our work-life balance policy aims to enhance employee's satisfaction and productivity by allowing flexible working hour. It will also help to attract and retain people. We are willing to support our employees to actively participate in community activities and accomplish personal responsibilities and development outside work.

7. Finance

7.1 Capitalization

Singapore has a host of grants that support local start-ups and NUS_Singapore can capitalise on these resources. NUS_Singapore has acquired a start-up budget of S$67,324.00 from Science Dean's Office of NUS (Table 2). With this starting capital, NUS_Singapore can apply for the ACE Startups grant provided by SPRING Singapore. This grant provides funding via matching S$7 to every S$3 raised by the startup to a cap of S$50,000. With the initial capital of S$67,324 raised, NUS_Singapore will be able to receive an additional S$50,000 should our application for the grant be successful. To further defray the costs, NUS_Singapore can also apply for the Research Incentive Scheme for Companies (RISC) provided by the Singapore Economic Development Board. Under this scheme, the government can provide 30-50% financial support in expenditure related to manpower, equipment and consumables. Alternatively, NUS_Singapore can also apply for the Technology Enterprise Commercialisation Scheme (TECS) by SPRING Singapore. As NUS_Singapore would have proven the scientific validity of the product, it will be considered under the Proof-Of-Value Project which supports the further development of a working prototype and validation of the commercial merit of an established concept. TECS will finance up to 85% of the qualifying costs for each project to a maximum of S$500,000.

Table 2: Funding from Science Dean's Office.

Item		Amount estimated (SGD)	Amount funded (SGD)
Fixed costs	iGEM registration fee*	6,087.49	6,500.00
	Jamboree participation*	12,197.25	13,000.00
	Travelling expenses	15,800.00	11,000.00
Variable costs	Consumables and reagents		36,824.00
Total			**67,324.00**

As seen from Table 2, most of the cost with regards to the registration and Jamboree participation will be covered, with only a positive difference for the travelling expenses that will be borne by NUS_Singapore.

In addition to the initial capital and various grants, NUS_Singapore has also approached sponsors such as New England Biolabs (NEB) and Integrated DNA technologies (IDT) who are willing to subsidise the costs of reagents and materials required, thus reducing production costs further.

7.2 Fixed and variable costs

Table 3 summarises the fixed and variable costs involved in this project. The fixed costs are mainly administrative fees that involve participation in the iGEM and the funds needed to register and test our product. Apart from clinical trials, medical products sold in Singapore would have to apply for a license and this contributes greatly to our fixed cost. According to GN-13: Guidance on the Risk Classification of

General Medical Devices, Revision 1.1 (Health Sciences Authority, 2014), the RIOT system will fall under the Class D medical device which is manufactured from a microbial or recombinant origin and require a full evaluation fee.

Table 3: Fixed and variable costs.

Item		Amount (SGD)
Fixed costs	iGEM registration fee*	6,087.49
	Jamboree participation*	12,197.25
	Travelling expenses	15,800.00
	Accommodation	2,860.00
	Clinical trial	30,000.00
	Registration process at Health Sciences Authority (HSA): - Evaluation fee - Annual retention fee at HSA - Annual dealer's licence fee at HSA as manufacturer	 11,400.00 120.00 1000.00 **12,520.00**
	Sales and marketing	2,000.00
		81,464.74
Variable costs	Manufacturing (consumables and reagents)	36,824.00**
Total		**118,288.74**

*Prices were calculated based on the prevailing exchange rate of USD1 = SGD1.35

** Variable costs are subjected to change at the end of the project

The laboratory space is provided free of charge during the research and development phase.

7.3 Projected income for the first year

Based on Table 4, there was a sharp rise in the number of Singaporeans diagnosed with cancer from 2010 to 2014 in Singapore. This reflects the growing demand for an accurate and cost-effective diagnostic and hence we hope to make it more convenient for people to have cancer screening tests with our product at an affordable price to meet the urgent society need to control this disease and improve public health. Based on this huge demand, we calculate the projected profit and loss for the company in Table 5. We assume that the RIOT system has already passed the clinical trials and is feasible as an alternative to

current cancer diagnostics. There is a myriad of cancer diagnostic tests that vary based on type (blood test for cancer markers, machinery-based such as a mammogram etc) and correspondingly, there is a range of prices for these tests, ranging from S$50 to S$1000[1]. With the specificity and non-invasive characteristics of the RIOT system, we aim to sell each diagnostic test at S$100.

Table 4: Number of Incident Cancer Cases by Year of Diagnosis, 2010-2014.

Year of diagnosis	2010	2011	2012	2013	2014
No. of notifications	11,431	11,726	12,295	12,651	13,416

Source: National Registry of Diseases Office, 2015

Due to the flexibility of the RIOT system, it is possible to extend its use beyond being a diagnostic to a complete drug delivery system. If this is the case, the costs will be adjusted based on the cost of production and the market demand. Generally, subsidized patients in Singapore pay about S$3000 - S$7000 per treatment for drug targeted therapies such as Herceptin and Avastin. Herceptin is used to treat early and late-stage breast cancer and late-stage gastric cancer while Avastin is used to treat late-stage colorectal cancer, late-stage non-small cell lung cancer, late-stage ovarian cancer, late-stage breast cancer, glioblastoma multiforme (a type of brain cancer) and late-stage renal cell carcinoma (Teo J, 2014). The original price for this category of drugs can cost up to $6000 to $9,800 per patient per month (Easton J., 2015). Based on the market price, the cost of our future drug delivery system is planned at S$4000 per treatment before subsidy. Depending on subsidy and insurance policy, patients can enjoy more affordable price. As there are no precedents with regards to the doses required for patients using bacterial-based cancer therapy, we have decided to look towards the typical number of doses required for a chemotherapy cycle.

In order to estimate the projected number of units of our bacteria that we can sell, we looked to our preliminary market survey and it revealed that 25% of the respondents indicated that they are completely receptive to novel bacterial therapies. Therefore, we project a modest 25% of the number of cancer cases diagnosed in 2014 could be our potential market. Therefore, our target sales for the first year is 3000 units. With marketing effort and increasing awareness of publics about the importance of health screening, the sales can be increased in subsequent years which may help us to bring down the costs to benefit the public health. The current sales target of the RIOT system as a diagnostic device will generate the net income before tax for the first year is expected to be S$181,711.26.

S$100,000 will be used to invest in the production, as well as research and development activities of next year and the balance will be shared among the stakeholders. This profit will likely increase in the following years because our system is scalable at lower cost and we only need to pay annual retention and license fees instead of a substantial amount of registration fees.

[1] Sata CommHealth (2014). *Health Screening Packages* http://www.sata.com.sg/wp-content/uploads/2014/09/2014-Health-Screening-Package-brochure-price-GST-WEB.pdf

Table 5: Projected income statement for the first year

Revenue	
Sales*	300,000.00
Total revenue	300,000.00
Expenses	
Cost of goods sold	36,824.00
Clinical trials	30,000.00
Medical device registration	12,520.00
Administrative	36,944.74
Sales and marketing	2,000.00
Total expenses	118,288.74
Income before Tax	**181,711.26**

*assuming that 3000 units of our product are sold

8. Risk Assessments and Critical Assumptions

8.1 Product Risk

There is a risk that our desired bacterial therapy product cannot be produced, or does not have the desired effect. Safety regulations and procedures must be followed when producing the bacteria. There may be an escape of bacteria/genetically modified organism into the environment. Solutions to minimise risk include animal and clinical trials, and following safety protocols.

8.2 Market Risk

The use of bacteria as a diagnostic tool is a relatively new concept, with some advances made in the field of detecting liver metastases via the urine (Danino *et al.,* 2015). Current diagnostic tools for cancer are mostly targeted towards a group of cancer e.g. breast magnetic resonance imaging (MRI) only works in detecting the presence of a tumour in the breast, and a diagnostic tool that is able to detect a broad range of cancer is lacking.

At the same time, it is likely that doctors will prefer conventional therapies that have been tried and tested, and may be adverse to the use of bacterial diagnostic tools. This may influence the attitudes of patients as they often listen to the opinions of their doctors. Also, doctors who had received their medical education would not have learnt about using bacteria as a diagnostic tool, therefore there may be some resistance to applying this new technology to patients. Solutions to minimise risk include market research and outreach to the general public to inform them of this potential diagnostic tool.

8.3 Competition Risk

There has been research done on the use of bacteria as a diagnostic for liver cancer and diabetes. However, as the research appears to still be for a specific cancer type/disease, it appears that our idea for a broad-spectrum cancer diagnostic faces little market risk.

8.4 Assumptions

One assumption is that there is a need for our product. There is a need for novel and new broad-spectrum diagnostic tool for cancer /assumption that a single conventional diagnostic test is insufficient to diagnose all forms of cancer. Another assumption would be that the patients are willing to undergo treatment. There is an assumption that patients will be willing to pay for the new diagnostic tool for cancer which may be more costly than conventional diagnostic methods. Also, another assumption would be that we can scale the product to meet the market's demand at an efficient cost.

As the bacterial therapy is a relatively new concept of diagnosis for cancer, there is likely to be apprehension towards the usage of this tool over conventional diagnostic methods like breast magnetic resonance imaging (MRI) or colonoscopy. Thus, it is likely that when this diagnostic tool is first introduced into the market, sales will be slow. However, if we have clinical trial results to prove that it will likely work, then it is likely that the reception will be more welcoming since this allows for detection of a broad range of cancer types instead of just specific types of cancers.

9. Policies or regulation surrounding selling the bacteria

In order to create the intended bacterial therapy, there are a few procedures that have to be done. After the initial testing phase on cell lines, the therapy will have to be tested on animals. In order to do so, a license has to first be obtained from the Agri-Food and Veterinary Authority of Singapore (AVA) under the Animal & Birds (Care and Use of Animals for Scientific Purposes) Rules in order to carry out experiments on animals. The Guidelines set by National Advisory Committee for Laboratory Animal Research (NACLAR) must also be complied with[2]. The NACLAR Guidelines are generally based on three principles: Replacement, Reduction and Refinement. 'Replacement' means to replace animals with alternative methods whenever possible, while 'Reduction' refers to the number of animals involved in the trials. In order to minimise the effects of the trials on animals, there should also be a 'Refinement' of the project and techniques used. The Guidelines will have to be followed in order to qualify for a license from AVA. An Institutional Animal Care and Use Committee (IACUC) will also have to be established, and they will oversee the evaluation of animal care and use programmes in an institute, ensuring that the use of animals for scientific purposes and the animal experimental procedures used are in compliance with the Guidelines set by NACLAR. Appropriate trainings will also have to be conducted before carrying out any experiments involving animals.

If the bacterial therapy works on animals, the next step would be to conduct the tests on patients with cancer via clinical trials. Non-clinical studies that provide toxicology information which help to support clinical trials should also be conducted, and they should comply with the Good Laboratory Practice (GLP) system that helps to assure regulatory agencies that the non-clinical safety data are accurate and reliable for the purpose of risk assessment[3].

Conduct of clinical trials in Singapore is regulated by the Medicines Act 1975 and the Medicines (Clinical Trials) (Amendment) Regulations 1998[4]. Before clinical trials can be conducted, the Principal Investigators (PI) must first obtain both ethics and regulatory approval. The ethics approval has to be obtained from the hospital's Institutional Review Board (IRB), while the regulatory approval has to be obtained from the Health Sciences Authority (HSA). In order to ensure that the conduct of clinical trials follows internationally acceptable ethical and scientific standards, the Singapore Guideline for Good Clinical Practice (SGGCP) has to be followed, and investigators will have to fulfil the SGGCP training requirements as specified by the IRB[5] [6].

[2] http://www.ava.gov.sg/explore-by-sections/pets-and-animals/animal-in-scientific-research/naclar-guidelines

[3] http://www.hsa.gov.sg/content/hsa/en/Health_Products_Regulation/Clinical_Trials/Overview/FAQ.html#c1b

[4] http://www.hsa.gov.sg/content/hsa/en/Health_Products_Regulation/Clinical_Trials/Overview/Regulatory_Guidelines/Guideline_on_Application_for_CTC.html

[5] https://www.research.nhg.com.sg/wps/wcm/connect/romp/nhgromp/trainingeducation/coursecategories/singapore+guideline+for+good+clinical+practice+course

[6] http://www.hsa.gov.sg/content/hsa/en/Health_Products_Regulation/Clinical_Trials/Overview/FAQ.html#e3

In order to obtain the regulatory approval, evidence that the treatment is acceptably safe and adequate levels of protection are provided for the participants must be shown. If the regulatory approval is obtained, a Clinical Trial Certificate (CTC) will be issued to the PI, and is valid for two years unless otherwise stated. The Singapore Guideline for Good Clinical Practice (GCP) that is based on international ethical and scientific quality standards must be abided by in order to ensure that the participants' rights and interests are adequately protected. The GCP also ensures that the safety and efficacy data obtained from the clinical trials are valid and accurate. Some example of some organisations that the RIOT system can work with in Singapore are SingHealth Investigational Medicine Unit (IMU) and Changi Trials & Research Unit (CTRU). A Trial Status Report will also have to be submitted to HSA every 6 months (from the CTC approval date), and whenever there are significant changes to the status of the trial (e.g. termination of the trial site)[7].

Some guidelines that can be referred to would be the FDA CDER Guidance for Industry: Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers and EMA Guideline on Strategies to Identify and Mitigate Risks for First-In-Human Clinical Trials with Investigational Medicinal Products (Reference number: EMA/CHMP/SWP/28367/07) in order to determine the maximum safe starting dosage and reduce risks to patients/volunteers in the clinical trials[8].

If the bacterial therapy passes clinical trials, then the next step would be to register it as a medical device. In order to register the medical device, dealers of the RIOT system will have to be first registered with the Accounting and Corporate Regulatory Authority (ACRA), and product registration will also be required[9]. Under the HSA regulatory framework, our product is likely to be classified as a general medical device under the Risk Group D, as our devices are of a microbial origin[10], and registered as a single medical device[11]. In order to register the RIOT system under a general medical device, the ASEAN Common Submission Dossier Template (CSDT) form must be filled in[12]. A full evaluation will also have to be obtained for the RIOT system, and a letter of authorisation, list of configurations of medical devices to be

[7] http://www.hsa.gov.sg/content/hsa/en/Health_Products_Regulation/Clinical_Trials/Overview/FAQ.html#b6

[8] http://www.hsa.gov.sg/content/hsa/en/Health_Products_Regulation/Clinical_Trials/Overview/FAQ.html#c1a

[9] http://www.hsa.gov.sg/content/dam/HSA/HPRG/Medical_Devices/Overview_Framework_Policies/Regulatory_Framework/QUICK%20GUIDE%20TO%20MEDICAL%20DEVICE%20PRODUCT%20REGISTRATION%20AND%20LICENSING%20(PDF)_Nov%202015_Ver%203.pdf

[10] http://www.hsa.gov.sg/content/dam/HSA/HPRG/Medical_Devices/Overview_Framework_Policies/Guidances_for_Medical_Device_Registration/GN-13-R1.1%20Guidance%20on%20the%20Risk%20Classification%20of%20General%20Medical%20Devices.pdf

[11] http://www.hsa.gov.sg/content/dam/HSA/HPRG/Medical_Devices/Overview_Framework_Policies/Guidances_for_Medical_Device_Registration/GN-12-1-R2%20Guidance%20on%20Grouping%20of%20Medical%20Devices%20for%20Product%20Registration%20-%20General%20Grouping%20Criteria.pdf

[12] http://www.hsa.gov.sg/content/dam/HSA/HPRG/Medical_Devices/Overview_Framework_Policies/Guidances_for_Medical_Device_Registration/GN-17-D1.1%20Guidance%20on%20Preparation%20of%20a%20Product%20Registration%20Submission%20for%20GMD%20using%20the%20ASEAN%20CSDT.pdf

registered and CSDT will have to be submitted[13]. The application will have to be submitted via the Medical Device Information and Communication System (MEDICS) platform. Besides the product registration, a dealer's license is also required, and can be obtained via application in the MEDICS platform. A Client Registration and Identification Service (CRIS) Company Account is also required for carrying out electronic transactions with HSA. In order for us to manufacture the RIOT system in Singapore, a Manufacturer's License (ISO 13485 certificate) is required, and a renewal every 12 months is required[14].

After obtaining the approval for manufacturing the RIOT system, records have to be established and maintained to prove that the Good Distribution Practice for Medical Devices (GDPMDS) requirements are met. A GDPMDS certificate will have to be obtained from GDPMDS Certification Bodies from Singapore Accreditation Council. The Health Products Act 2007, which sets the framework for characterising health products and ensuring the standard of health products are up to par[15], and Health Products (Medical Devices) Regulations 2010 will also have to be complied with.

13 http://www.hsa.gov.sg/content/dam/HSA/HPRG/Medical_Devices/Overview_Framework_Policies/Guidances_for_Medical_Device_Registration/GN-15-R6.1%20Guidance%20on%20Medical%20Device%20Product%20Registration.pdf

14 http://www.hsa.gov.sg/content/dam/HSA/HPRG/Manufacturing_Importation_Distribution/Guidance%20documents%20for%20Industry/GUIDE-MQA-026-001.pdf

15 http://www.hsa.gov.sg/content/hsa/en/Health_Products_Regulation/Useful_Information_for_Applicants/Legislation.html#HealthProductsAct

10. Exit strategy

10.1 Need to identify potential buyers (pharma companies) of our bacteria

As we continue to monitor the market behaviour concerning RIOT system, we plan to sell our product when the technical operations – including upstream-to-downstream production, quality assurance and checks, commercial managements – are established and when its sales figure and profits are gaining great traction – such that our product valuation would approach its optimum, and garnering interest from companies might prove to be an accessible scheme.

Therefore, in the medium-to-long term, we would identify potential investors – including venture capitals and pharmaceutical companies. The Roche Group is the world's leading manufacturer of anti-cancer products, and Roche Diagnostics is also the world leader in in-vitro disease diagnostics. Novartis, Celgene, and Johnson & Johnson are ranked subsequently in terms of oncology sales (GlobalData) in the recent five years. Our RIOT system would be classified as a non-conventional in-vivo diagnostics tool, and we plan to approach these companies and assess if there is any interest alignment. In conclusion, we would like to sell our product to an established pharmaceutical company. Otherwise, if said company would like to collaborate with us in other manners, such as in extending the use of RIOT system beyond cancer diagnostics towards a complete drug delivery system, we would be keen to participate as well.

10.2 Acquisition Plan

Depending on how the potential investor/buyer value our product, we would frame our proposal accordingly – either based on the financial value or strategic value of RIOT system, or both. Our team feels that the strategic approach would hold more potential for collaboration, as we believe in the flexibility of our product to grow into a complete cancer drug delivery system. Conceptually, our molecular system can also be applied to diagnose and treat other biomarker-exhibiting diseases – such as rheumatoid arthritis (RA) and Alzheimer's disease – or to indicate environmental exposure in studies involving HPV or tobacco exposure. While further research and development would need to be done on the current system, our team believes that this growth opportunity is very pertinent.

For this acquisition plan, the total deal value would largely depend on the financial forecast of our product, based on the most recent market value data.

10.3 Collaboration Plan

Our team has already researched into the process of developing RIOT system from a diagnostics tool into a cancer therapeutic product, as well as having the blueprint of our new prokaryotic system in place. Considering the non-conventional aspect of our product, and the risks it inherently carries as an in-vivo diagnostics tool, however, we have decided to firstly segment our product capability into the detection phase; then assess the clinical safety, efficacy, as well as the suitability of our pricing for our patients. Should this detection phase prove to be a success, we would approach the aforementioned companies for sponsorships, or collaboration. Overall, as the potential for RIOT system is expansive, our team is sure of its value-add attribute to a pharmaceutical company focusing on cancer diagnostics and therapeutics.

11. Conclusion

NUS_Singapore is confident that we can achieve what is set out in our comprehensive business plan, meeting the sales forecasts and generating a profit of at least **$181,711.26** in our first year of operations. By launching our product, the RIOTsystem, we hope to revolutionise the medical diagnostic industry, bringing new breakthroughs to help cancer patients. With our system being very robust and flexible, more Research and Development into our system can easily bring the company into new industries of diagnosing other diseases or in the industry of therapeutics. The company has the potential to become a highly regarded resource in the local, regional, national and international markets. With the company's profitable revenue model, we have the potential to provide lucrative returns for potential investors. Providing that the company is able to acquire its funding requirements, The Company will be able to achieve operational success for many years to come.

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